NOTES ON THE DEVELOPMENT OF GORGODERA AMPLICAVA IN THE FINAL HOST

THERON O. ODLAUG

(From the Department of Biology, University College, New York University and the Marine Biological Laboratory, Woods Hole, Mass.)

Introduction

Loschge (1785) first reported flukes in the bladder of *Rana esculenta* and Zeder (1800) named them *Distomum cygnoides*. On the basis of the number of testes, Looss (1902) separated the bladder flukes of frogs into two genera, *Gorgodera* and *Gorgoderina*, the former genus possessing nine testes and the latter, two. Bladder flukes of frogs have been reported in North America by Leidy (1851), Bensley (1897), Stafford (1902), Cort (1912), Ingles and Langston (1933), and Ingles (1936). Seven species of gorgoderid worms have been described in North America: *Gorgodera amplicava* from *Rana clamitans, R. catesbeiana*, and *R. pipiens; Gorgodera minima* from *R. catesbeiana* and *R. pipiens; Gorgoderina simplex* from *R. catesbeiana* and *Bufo lentiginosus; Gorgoderina translucida* from *Bufo lentiginosus* and *R. virescens; Gorgoderina attenuata* from *R. catesbeiana* and *R. virescens; Gorgoderina multilobata* from *R. boyli* and *R. aurora; Gorgoderina aurora* from *R. aurora*. Krull (1934), who reported the principal stages in the life history of *Gorgodera amplicava*, found that the clam, *Musculium partumeium* served as the first intermediate host, the snail *Helisoma antrosa* as the second intermediate host, and the frogs *R. clamitans* and *R. catesbeiana* as the final hosts.

The first life history studies on bladder flukes were done by Ssinitzin (1905) who traced the development of *Gorgodera cygnoides, Gorgodera pagenstecheri*, and *Gorgodera varsoviensis*. He found that the cercariæ were of the cystocercous type, that they were produced in bivalve mollusks, and that the metacercariæ developed in the aquatic larvæ of insects. Lutz (1926) indicated the intermediate hosts through which species of *Gorgoderina* might pass in order to complete the life cycle. He found the cercariæ in two small bivalves, *Cyclas* and *Pisidium* or *Sphærium*, and the metacercariæ encysted in the esophagus of odonatan larvæ. The same author also stated that the parasites in the final host, which is some anuran, were often found in the ureters. He observed

that " En effet, les conduits efférents du rein contiennent assez souvent les formes jeunes. Dans le *Leptodactylus pentadactylus, Gorgoderina permagna* devient presque mûre et *Gorgoderina diaster* a été trouvée pleine d'œufs dans l'uretère de *Pseudis paradoxa.* Chez la *Rana palmipes* il y avait des exemplaires dans la vessie, mais le plus grand se trouvait dans l'uretère." Joyeux and Baer (1934) found three young *Gorgoderina* in the muscles of the ventral body wall of *Rana esculenta;* the worms were not encysted and appeared to be migrating in the muscles without provoking any reaction on the part of the host. They further observed that the genital apparatus was fully functional and that the uterus was full of eggs, some of which had reached the genital pore. These specimens they reported to be morphologically similar to the adult form of *Gorgoderina capsensis* which they described from the urinary bladder of *Rana esculenta.* They concluded that the trematodes may become adult before they reach their definitive habitat, the urinary bladder.

The present paper records the occurrence of *Gorgodera amplicava* in the kidneys of *Rana catesbeiana,* together with a brief description of the trematode, particularly of its reproductive system, in the final host.

Material and Methods

The material for the present study was obtained from the common bull frog, *Rana catesbeiana,* at the Marine Biological Laboratory, Woods Hole, Mass. In routine examination of frogs during the summer of 1936, a number of trematodes were observed in the kidneys. Accordingly, the excretory organs of fifteen frogs were dissected out for further study. No worms were found in the ureters. The kidneys were pressed between two glass slides in order to observe, if possible, the location of the flukes. This method did not prove successful because of the amount of blood present, so the kidneys were dissected, a small portion at a time, until the parasites were located. Twenty-four worms were thus removed, flattened under a cover-slip, and fixed in hot corrosive sublimate. All material was stained in paracarmine, and measurements were made on fixed and stained specimens.

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Observations

With but one exception, all of the worms found in the kidneys were sexually immature. In the examination of one frog, a sexually mature

worm was observed emerging from a white, cyst-like enlargement on the surface of the kidney. Upon fixing and staining, it was found to be a specimen of *Gorgoderina attenuata* and not a representative of the genus *Gorgodera*. The cyst-like structure possessed an opening at the top through which the fluke probably passed its eggs. This trematode was the only sexually mature specimen found in the kidneys; all the small, sexually immature worms were *Gorgodera amplicava*.

The smallest specimen of *Gorgodera amplicava* from the kidney measured 0.753 mm. in length and 0.255 mm. in width immediately posterior to the acetabulum; the largest one measured 1.68 mm. by 0.45 mm. Neither one was sexually mature. The smallest specimen from the bladder measured 1.76 mm. in length by 0.52 mm. in width. At this stage, the specimens were also sexually immature. Although the increase in size is not significant, comparison of the reproductive organs in the largest specimen from the kidneys with those in the smallest one from the bladder shows the latter to be much more nearly mature.

The series of worms ranging from the smallest individuals in the kidneys to the sexually mature forms in the bladder exhibits a gradually increasing development. In the youngest worms from the kidney, the genital anlagen, with the exception of the testes, appear as a sac-like mass of cells immediately posterior to the acetabulum (Fig. 1). In the next stage (Fig. 2), this mass has increased in size, branched into a right and a left lobe, and has also elongated posteriorly. The lobes then become separated from the common cell mass to form two discrete bodies, the future ovary developing from the left lobe and the vitelline follicles from the right one. At this stage, the vitelline follicles consist of a single mass of cells, later division resulting in the two groups of follicles, left and right, that are characteristic of the adult.

Gorgodera amplicava is definitely protandrous. All of the youngest individuals (stage shown in Fig. 1) taken from the kidneys possessed twelve well developed testes, five on the right side and seven on the left. According to Krull (1934), the testes are at first represented by a dense mass which later becomes separated into discrete organs. Originally, the testicular mass was a part of the common genital anlage, but because of the protandrous tendency of *Gorgodera*, the testes develop before the rest of the reproductive system. It appears that the testicular mass always subdivides into more than the nine parts which are characteristic of the adult. As stated above, the youngest specimens possessed twelve testicular masses; this number was reduced to ten in later stages (Figs. 2 and 3), and finally to nine, the number characteristic of the adult.

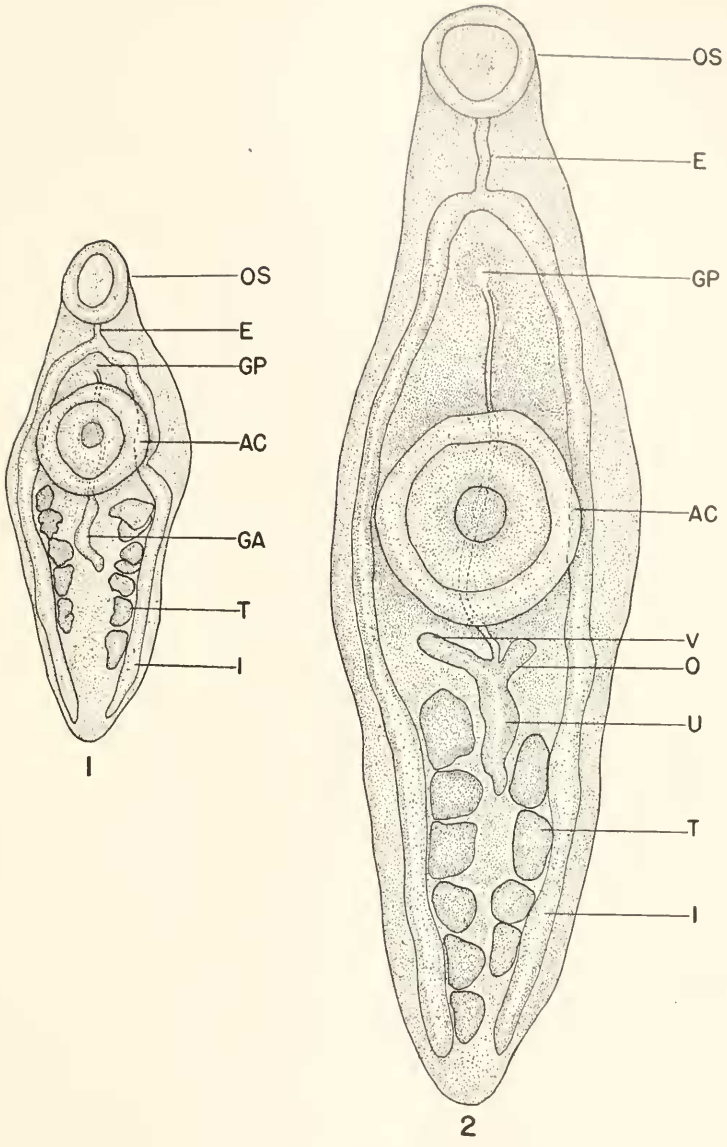

PLATE I

FIG. 1. Smallest form of *Gorgodera amplicava* from the kidney of *Rana catesbeiana*, ventral view. × 85.

FIG. 2. Largest form of *Gorgodera amplicava* from the kidney of *Rana catesbeiana*, ventral view. × 85.

AC, acetabulum.
E, esophagus.
GA, genital anlage.
GP, genital pore.
I, intestine.
O, ovary.
OS, oral sucker.
SV, seminal vesicle.
T, testis.
U, uterus.
V, vitelline follicle.

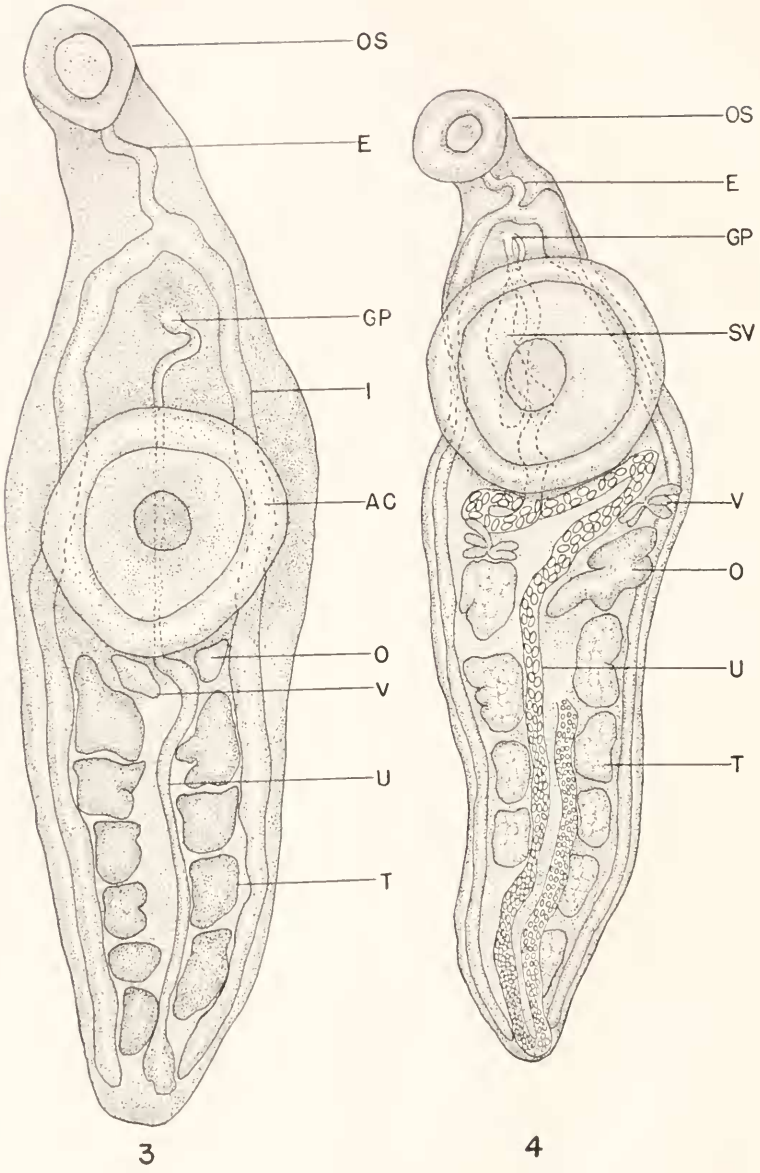

PLATE II

FIG. 3. Smallest form of *Gorgodera amplicava* from the bladder of *Rana catesbeiana*, ventral view. × 85.

FIG. 4. Adult *Gorgodera amplicava* from the bladder of *Rana catesbeiana*, ventral view. × 28.

Discussion

Krull (1934) stated: "In stained and mounted specimens the dense testicular mass shows little evidence of being subdivided into testes, and there is little separation into right and left part until maturity." In the present study, observations on the youngest individuals showed not only division of the mass into right and left components, but also twelve well developed testes; at this time, the female genital complex was as yet undifferentiated. It is probable that the original testicular mass may be subdivided into a variable number of components. In the writer's opinion, after the early division of the testicular mass, there is a coalescence of certain of the testes to form the definitive number.

The observations of Lutz (1926) showed that specimens of *G. permagna* may develop in the ureters of *Leptodactylus pentadactylus* and *G. diaster* in the ureters of *Pseudis paradoxa*. He also found gorgoderid worms in the ureters of *Rana palmipes*. Since these ducts open directly into the cloaca and are independent of the urinary bladder, it appeared that excysted metacercariæ, when they reach the cloaca, may pass either to the bladder or to the mesonephric ducts. Although Lutz himself made the statement: "On distingue *Gorgodera*, a testicules nombreux, de *Gorgoderina*, que n'en a que deux . . . ," it is impossible to determine from his report whether the species *G. permagna* and *G. diaster* are members of *Gorgodera* or *Gorgoderina*, since he referred them first to one genus and then to the other.

The discovery by Joyeux and Baer (1934) of sexually mature specimens of *Gorgoderina capsensis* in the abdominal muscles of *Rana esculenta* is difficult to interpret and introduces further complications in the life history of the frog bladder flukes. It is not impossible that abnormal physiological conditions, resulting from inanition of the hosts, were responsible for migration of the worms to the muscles.

In infection experiments with the definitive host, Krull (1934) fed a number of metacercariæ of *Gorgodera amplicava* to *R. clamitans* and *R. catesbeiana* and later (21 days to 2 months) recovered adults from the bladder. He stated that "On the basis of a rough estimate derived from the infection experiment, it appears that about one-fifth of the number of metacercariæ fed to a frog may be recovered from the bladder upon subsequent examination." About eighty per cent of the specimens fed were not accounted for. No explanation was offered for the disappearance of such a large number of worms, but from the present work it appears probable that a number of them might have been found in the kidney ducts in various stages of development.

Significance must be attached to the fact that developing *G. amplicava*

were found regularly in the kidneys. They appeared too often in that location to be merely an accidental infestation, and, furthermore, no sexually mature forms of this genus were found in these organs. It appears probable that the adult *Gorgodera* is too large to migrate up the ureters and for that reason it would be but logical to find only the young stages in the kidneys. On the other hand, the presence of a sexually mature *Gorgoderina attenuata* in the kidney and the fact that *G. attenuata* is much larger than *Gorgodera amplicava* may indicate that it is possible for the adults to migrate to the kidneys. However, the single specimen of *Gorgoderina attenuata,* which was found in the kidney, may have migrated there as a young form and developed to maturity in that location. The fact that no adult specimens of *Gorgodera amplicava* were found in the kidneys and the discovery of a developmental series of worms grading from the smallest in the kidneys to the adults in the bladder, suggest strongly that *Gorgodera amplicava* normally passes a stage of its life cycle in the kidneys before returning to the bladder to become sexually mature.

There seems to be no correlation between the number of worms found in the kidneys and those present in the bladder. In some of the frogs examined, all the specimens were found in the bladder and none were taken from the kidneys; in other instances the reverse was true. In certain frogs, some worms were found in the bladder and others in the kidneys. In every case, however, the specimens from the kidneys were sexually immature while those from the bladder were either adults or individuals in various stages of late development. Yet even here, the smaller forms taken from the bladder were in a more advanced stage of development than those from the kidneys; forms as young as the one represented in Fig. 1 were never found in the bladder. Accordingly, it seems clear that the time of infestation and the rate of development of the parasite may explain the number of worms present in each of the two locations.

Summary

Additional stages in the development of *Gorgodera amplicava* in the final host, *Rana catesbeiana,* are reported. Developmental stages have been found in the kidneys and their ducts and a series of changes in the development of the genital organs is described.

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